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SPERMATOGENESIS OF THE HORSE WITH SPECIAL REFERENCE TO THE ACCESSORY CHROMOSOME AND THE CHROMATOID BODY.

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CONTENTS.

I. INTRODUCTION.

Many interesting things were observed in this study on the spermatogenesis of the horse, but the two points of especial interest and importance are; firstly, the occurrence of a large accessory chromosome, and secondly, the presence of a much smaller though very conspicuous body comparable to the chromatoid body as described by Professor E. B. Wilson ('13) in *Pentatoma*. While the significance of the chromatoid body is problematical, it is a body of extreme interest in this connection on account of its deceptive resemblance to an accessory chromosome. Were it not for the fact that its entire history can be

followed out it might lead to serious misinterpretations. Since the occurrence of the chromatoid body in the horse is so constant and its behavior so distinct, and furthermore, since this is the first case among the vertebrates where such a body has been studied in full detail, it is dealt with at some length in this paper.

The significance of the accessory chromosome is of course obvious. It was shown beyond doubt that sex in the pig is determined by such elements (Wodsedalek,' 13). And while embryological material of the horse is not at present available to enable a similar extended study, the presence and unquestionable behavior of the accessory chromosome giving rise to a dimorphic condition among the spermatozoa of this mammal, nevertheless, lend additional support to the chromosome theory of sex determination. The spermatogenesis of the horse resembles to a certain extent the spermatogenesis of the pig (Wodsedalek, '13), and for the purpose of avoiding too much repetition it is treated in a comparative way in the present study.

This investigation was started in the zoölogical laboratory at the University of Idaho, but the main bulk of the work was done at the Wisconsin Biological Station at Madison. And I wish to thank the zoölogy department of the University of Wisconsin for the liberal use of their laboratories, apparatus and material, and the many other courtesies extended me during the summer of 1914.

II. Material and Methods.

The material studied, mainly, was obtained from a horse about a year and a half old. Immediately after the testes were removed from the live animal, small pieces were placed in Bouin's and Gilson's fluids. Sections from various parts of the testes were made from four to ten microns thick, and the material fixed in Bouin's fluid and stained with Heidenhain's iron hematoxylin with acid fuchsin as a counterstain, as in the case of the pig, proved to be the most satisfactory.

Material from an animal about a year old was also studied; but while all the stages including the mature sperm could easily be identified in this material, the chromosomes were very difficult to count on account of being too closely aggregated or lumped

together. The finer details of the cells, too, were not as easily made out as in the other material, this being undoubtedly due to the fact that the material was not fixed until about an hour after it was removed from the animal. The chromatoid body, however, was very distinct and could be traced throughout its entire history the same as in the more favorable material. The accessory chromosome, too, could easily be identified, especially through the first spermatocyte division.

III. General Arrangement of the Germ Cells.

The structure of the testes of the horse differs from the pig in that the seminiferous tubules as well as the corresponding cells in the various degrees of development are much smaller and the interstitial cells are much fewer in number. The continuous network of connective tissue walls is present, but the chambers formed by this network and filled with coiled tubules are much larger in the horse and, therefore, a section through one of the chambers as a rule reveals many more sections of the tubules. These chambers in the horse testes do not show the same regularity in size as is the case in the pig testes. In some cases a group of over a hundred sections through the tubules are surrounded by the connective tissue wall and then again a count of only a dozen or so can be made. The arrangement of the cells in the tubules is similar to that of the other well-known mammals, particularly the pig.

IV. Spermatogonia.

As a rule the spermatogonia lie in a single layer next to the wall of the tubule, though occasionally some of the cells are crowded out. At times the cells are far apart, in which case they are flattened out on the tubule wall. The cells also differ considerably in size and appearance, depending on the stage of development they are in (Figs. 1–3).

During the resting stages a large nucleolus is invariably present. As a rule it assumes a somewhat heart-shaped appearance; especially is this true in the larger cells and in those in which the chromosomes are beginning to form. A much smaller spherical nucleolus also appears to be fairly constant (Figs. 1

and 2). Other nucleoli varying considerably in size, shape, and number also appear in some of the cells (Fig. 2).

Before the chromosomes begin to form the cells increase greatly in size (Fig. 2). At the conclusion of the resting stage numerous large chromatin granules appear which arrange themselves along fine threads in an entangled mass. The chromosomes soon become distinct and while, as a rule, a count is impossible on account of the overlapping and massing together of the chromosomes, [the mitotic stages were abundant and many distinct counts could be made. Thirty-seven chromosomes appear in the late prophases of the spermatogonial division (Figs. 4 and 5). Thirty-six of these are variously shaped, mainly oblong, and differ somewhat in size. One which is much larger is, as a rule, somewhat triangular or heart-shaped. This is the accessory chromosome and is the same thing as the large nucleolus which appears in the resting stages. That is certain, as the body can easily be traced through the various stages of growth. This condition is similar to that found by Guyer ('10) in man, and Wodsedalek ('13) in the pig. Ordinarily about two thirds of the chromosomes arrange themselves in a ring which encircles the remaining one third. The accessory chromosome may be found anywhere within the mass, and occasionally occurs outside of the main ring, but never far removed from the other chromosomes. During division each chromosome divides in two. The accessory as a rule divides a little in advance of the other chromosomes (Figs. 6 and 7).

The spermatogonia in this, as well as other stages, vary somewhat in size (Figs. 6 and 7). In the smaller cells the cytoplasm appears denser and the chromosomes are more crowded together.

V. Primary Spermatocytes.

1. *Resting Stage.*

The primary spermatocytes arising from the final spermatogonial division in the early resting stage are usually smaller than the spermatogonia immediately preceding and during the division stages. After the disintegration of the chromosomes the nucleus appears much clearer than it does in the later growing stages. The large nucleolus is again very conspicuous and easy

to distinguish from other nuclear bodies when such are present (Figs. 8–11). The small spherical nucleolus again appears to be fairly constant, though at times it is difficult to distinguish it from the other bodies.

2. *Synizesis and Growth Period.*

After a brief period of rest the cells begin to increase in size. For some time the nucleus appears much the same as it does in the resting stage of the spermatogonia (Fig. 8). Later it becomes more granular and the linin fibers become more distinct (Fig. 9). Soon after, the chromatin threads become massed in the center of the nucleus (Fig. 10), and later the nuclear wall expands and the entire mass passes to one side of the nucleus, leaving a large clear area in the remaining portion (Fig. 11). This condition is much the same as in the pig except that in that animal the nucleoli were invariably found within the mass of threads and in a position nearest to the nuclear wall, while in the horse the nucleoli are almost invariably within, or next to the clear area (Fig. 11). The nuclear wall in this stage is often very irregular, especially next to the clear portion of the nucleus.

Shortly after the collapse of the chromatin material, the threads pair and appear in about half the original number and twice as thick (Figs. 10–12). There is considerable evidence that pairing of the threads takes place by parasynapsis, and nothing was observed which would indicate that it takes place otherwise; but this phase of the problem demands more study and no positive statement can be made in regard to it at this time. The entire mass of threads then moves toward the center and the large clear area disappears (Fig. 12). The large nucleolus passes toward the periphery of the nuclear wall and the threads soon become evenly distributed. Then follows the period of growth during which time both the nucleus and cytoplasm increase greatly in size (Figs. 13 and 14). The chromatin threads and the large nucleolus also increase considerably in size. It is between the synaptic stage and the fully developed spireme stage that the chromatoid body makes its appearance (Figs. 12–14).

3. *Reduction Division.*

Nineteen chromosomes appear in the late prophase or early metaphase stages of the primary spermatocyte (Figs. 15–18). Eighteen of these are the ordinary chromosomes or autosomes and the other is the accessory chromosome. The accessory in this case is practically always found outside of the main mass of chromosomes, either in close contact with them (Figs. 16 and 18), or a short distance away (Figs. 15 and 17). The large size of the eighteen autosomes which are about four times the size of the chromosomes in the spermatogonia indicates that they were formed by the growth and pairing of the thirty-six autosomes found in those cells, while the accessory remains unpaired, making a total of nineteen.

In these cells as in the case of the spermatogonia the chromosomes are frequently bunched together, making an accurate count difficult and often impossible. However, mitotic stages particularly of the first and second spermatocyte divisions were very numerous and among the thousands of cells in mitosis examined several hundred definite counts were made. Figs. 19–29 show the accessory in characteristic positions in the metaphases of division of the primary spermatocyte. The heart-shaped body always passes toward one pole in advance of the other chromosomes and frequently may be found at the pole before the other chromosomes have divided (Figs. 28 and 29). The chromatoid body which is spherical in shape and much smaller than the accessory is also invariably present and very conspicuous. As a rule it is in the spindle, and in a large majority of the cases goes in the direction opposite from the accessory (Figs. 21, 22, 23, 24, 25, 27 and 28), though this behavior is by no means constant, for occasionally it is found with the accessory on the same side of the equatorial plate (Figs. 20, 26 and 29).

When the large, apparently quadrivalent chromosomes divide, the resulting chromosomes are somewhat larger than the chromosomes of the spermatogonia. Immediately after the chromosomes divide they unite in twos (Fig. 29) so that at the time of their arrival at the poles they do not number eighteen, but only nine or exactly one half that number (Figs. 30–35). Additional proof that such a second pairing of the chromosomes occurs lies

in the fact that the resulting nine chromosomes are not one half the size of the original eighteen chromosomes of these cells, but exactly of the same size and apparently quadrivalent. This quadrivalent nature becomes obviated after the division of the secondary spermatocyte, where the resulting chromosomes are bivalent. The primary spermtocyte division is undoubtedly the reduction division and, speaking in terms of univalence, one of the resulting secondary spermatocytes receives eighteen chromosomes and the other eighteen plus the accessory. In terms of bivalence the one type of secondary spermatocytes receive nine chromosomes and the other nine plus the accessory (Figs. 30–33).

VI. Secondary Spermatocyte.

No resting stage occurs in the secondary spermatocyte, a condition similar to that frequently found in the spermatogenesis of the pig. The second pairing of the chromosomes also takes place here as it does in the pig (Wodsedalek, '13), man (Guyer, '10), and opossum (Jordan, '11). In the pig, however, this pairing takes place much later, never before the cell is completely divided. The secondary spermatocytes divide soon after they are formed and not infrequently the spindles are formed in the two cells resulting from the first spermatocyte division while they are still in close contact. Nine chromosomes arrange themselves in the equatorial plate for division in the one type of secondary spermatocyte (Figs. 42–45), and nine plus the accessory in the other (Figs. 34–38). All of the chromosomes, including the accessory when it is present, divide in these cells (Figs. 36–47). The accessory usually lies a little to one side of the other chromosomes (Figs. 34 and 35), and again, as in the spermatogonia, divides a little in advance of the other chromosomes (Figs. 36–38). This may be due to the partial separation of the two halves of this body even long before the other chromosomes line up for division in this stage (Figs. 21–33). The heart-shape it assumes during the later stages of the primary spermatocyte division and retains during the secondary spermatocyte, is no doubt due to a partial separation at one end of the two components. The chromatoid body remains very conspicuous (Figs. 35–55).

VII. Spermatids.

The division of the secondary spermatocytes gives rise in the one case to spermatids containing nine chromosomes (Figs. 46, 55 and 56), and in the other case nine plus the one accessory or ten chromosomes (Figs. 39–41). All of the chromosomes except the accessory are bivalent in nature (Figs. 23–41, 54–56), so that in reality we have the equivalent of eighteen chromosomes in the one kind of spermatid and eighteen plus the accessory in the other. All of the foregoing evidences indicate that eighteen is the reduced number of chromosomes.

The accessory is usually out of the main mass of chromosomes (Figs. 40 and 41). Soon after the secondary spermatocyte divides the chromosomes become massed together and the nuclear wall begins to form (Figs. 57–59). In the resting stage half the spermatids contain a large nucleolus which is the same thing as the accessory chromosome, since it can be traced through all the stages in the formation of the nucleus (Figs. 63–65). The other half of these cells lack such a body (Fig. 62). In some cases this nucleolus persists in the developing stages of the spermatozoön (Figs. 72 and 73). Especially is this true in material which has not been destained too much. In favorably stained material the centrosome surrounded by a clear layer can be seen within the centrosphere (Figs. 64–66). The chromatoid body is still very distinct (Figs. 55–67).

VIII. Development of the Spermatozoa.

The development of the spermatozoön in the horse is essentially the same as the development of the spermatozoön in the pig (Wodsedalek, '13). The centrosome surrounded by a clear area emerges from the sphere (Fig. 67) and soon divides into two spherical bodies (Fig. 68). The anterior one comes in contact with the nuclear wall, while the posterior one which remains spherical passes down the developing axial filament (Figs. 69, 70, 71, 73, 74). This posterior body which is quite small never assumes the shape of a ring as it does in the pig. It passes far down the filament and often no trace of it is left (Fig. 79). Then again it retains a size just enabling detection (Fig. 73). As a rule, however, a sufficient amount of it is left to be sloughed off

as in the case of the pig (Figs. 74, 75, 77 and 84). The chromatoid body is, in rare cases however, also seen on the filament and in such cases apparently fused with the posterior centrosome (Figs. 71 and 76). It is invariably sloughed off before the spermatozoön is fully developed (Figs. 77 and 86).

Shortly after the centrosome divides the nucleus begins to elongate and at the same time migrates toward one end of the cell, so that soon practically all of the cytoplasm is found at the posterior end of the developing sperm (Figs. 67–75). As the acrosome-end of the nucleus comes in contact with the cell-wall no break in the latter is ever noticeable, and the apparent backward pull exerted by the mass of cytoplasm causes the cell-wall to become closely applied to the nuclear wall where it undoubtedly persists as an additional covering of the sperm-head (Figs. 73–79). This supposition that the cell-wall forms an additional covering of the sperm-head is based on two observations; firstly, there is no evidence that the head penetrates the cell-wall, and secondly, the covering of the sperm-head is much thicker after the entire mass of cytoplasm lies at its posterior end (Figs. 73–79). This fact gives one the impression that the distinctly noticeable change in the thickness of the head covering is brought about by the fusion of the two walls. It is also obvious that the cell-wall is not entirely consumed in forming the external covering of the head of the sperm, for it can always be seen surrounding the anterior portion of the axial filament and extending far down into the mass of cytoplasm which is apparently squeezed out of it and about to be thrown off (Figs. 77–79). In the final stages it becomes closely applied to the axial filament and one may safely conclude that the axial envelope is at least partly formed by the portion of the cell-wall extending down from the head (Figs. 77, 79 and 85). This same condition was found to exist in the pig.

When the developing sperms reach the stage represented in Fig. 73 they become attached in clusters to the large nurse cells. As the sperms develop the cytoplasmic mass of the nurse cells decreases. Just as the mass of cytoplasm is being thrown off by the developing spermatozoa, the latter leave the nurse cells and become embedded in the layer of cytoplasm composed of the

cast-off masses, apparently nursing on the material so that little of it, if any, goes to waste.

Every stage in the sloughing off of the cytoplasmic mass can easily be observed (Figs. 77–86). When these masses of naked cytoplasm are completely sloughed off they assume a rounded shape and if the chromatoid body is present they might, at first sight, be mistaken for minute cells with the chromatoid body as the nucleus. And I feel that Wilson ('13), in speaking of this condition in *Pentatoma*, is absolutely correct when he says, "I also think it probable that the bodies that have been described as 'degenerating cells' in the late spermatid-cysts by some observers are identical with the protoplasmic balls here described."

Among the cast-off balls four different types can be observed (Figs. 80–83). One type contains a small body which apparently is the remnant of the posterior centrosome (Fig. 80); another type is clear and one is led to believe that in such a case the centrosome was entirely consumed (Fig. 81); another shows the same condition regarding the small body but contains the chromatoid body (Fig. 86); and still another contains both the chromatoid body and the much smaller centrosome remnant (Fig. 82). Later when the spermatozoa are fully developed the roundish masses become irregular in shape and finally begin to disintegrate. The disintegration is characterized by the breaking-up of the masses into small particles and by the appearance of many deeply staining bodies and globules which vary considerably in size (Fig. 83).

Occasionally in the last stages of the disintegration of the cytoplasmic material and also when the material entirely disappears there may be seen small, deeply staining bodies identical in size and appearance to the chromatoid body and one is led to believe that it is the same thing (Figs. 83 and 84). If it is the same thing the fact throws some light on its durable consistency.

The mature spermatozoön in general resembles that of the pig, except that it is smaller, and the head is thinner at the anterior end and thicker at the posterior end. The entire nucleus enters into the formation of the head and the contents become homogeneous and intensely staining.

IX. Variation in Size of Adult Spermatozoa.

The spermatozoa of the horse like those of the pig vary considerably in size and many careful measurements show that they, too, are of two distinct types, the one being much larger than the other. Mature specimens which were free in the lumen of the tubule and parallel to the objective, were selected at random and outline sketches of six hundred heads enlarged (× 2,000) were made with the aid of a camera lucida. The lengths of the sketches were then measured and recorded in quarter millimeters. It can be seen from Fig. 1 in the text that two separate types of

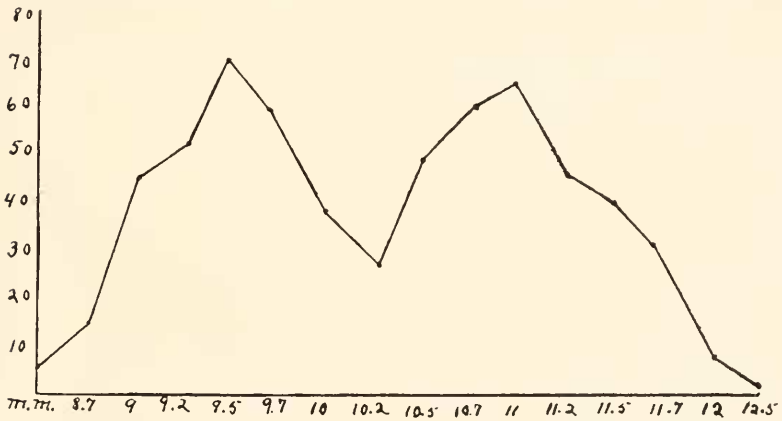

Fig. 1. Diagram showing the variation in size among six hundred mature horse spermatozoa. Figures at the left give the numbers of individuals belonging to each type. Figures at the bottom give the lengths of the heads of the spermatozoa in millimeters, magnified two thousand times.

spermatozoa exist; the greatest number of the one kind measuring 9.5 mm., and of the other 11 mm. I think it is safe to assume that this dimorphic condition in the size of the mature spermatozoa is due to the accessory chromosome. The increased size in the one type is due presumably to the presence of that element.

A similar dimorphic condition was found to exist among the spermatozoa of the pig; one type measuring from 11 to 12 mm., and the other from 14 to 14.5 mm. (Wodsedalek, '13). Size dimorphism also exists in the adult spermatozoa of *Anasa tristis* (Faust, '13). This of course is exactly what would be expected

since the spermatogenesis studies of this form indicate that one half of the spermatozoa receive one more chromosome each than the other half (Paulmier, '99).

X. Migration of the Developing Spermatozoa.

In the very beginning of the transformation of the spermatids into spermatozoa when the acrosome takes a position on the nucleus opposite the dividing centrosome (Fig. 68), the anterior end of the sperm-cell which bears the acrosome, invariably points in the direction of the tubule wall and the Sertoli or nurse cells. Long before the tail is sufficiently developed to aid in locomotion these sperm-cells move a short distance and come in contact in bunches with the nurse cells. There apparently exists some attraction between the nurse cells and the nuclei of the sperm cells in that stage of development. In this first stage of migration only the nucleus appears to be attracted while the cytoplasm exhibits a tendency to remain in place. The fact that the cytoplasm does have a tendency to remain in place while the nucleus or sperm-head moves forward undoubtedly accounts for the posterior position that the entire mass of cytoplasm assumes with respect to the sperm-head (Figs. 69–79). This change in position of the cytoplasm to the posterior end of the developing cell occurs simultaneously with the migration of the cell, which is further evidence for such an assumption. The nucleus is apparently attracted with sufficient intensity to enable it to pull the entire cytoplasmic contents after it to a certain extent. In the later stages the movement of the sperm-head deeper into the cytoplasm of the nurse-cell is probably facilitated by the flagellum-like motion of the filament which extends a considerable distance out of the cell (Figs. 75–79). Later, when the spermatozoa are almost fully developed and slough off the balls of naked cytoplasm (Figs. 85 and 86) they back out away from the nurse-cells, becoming embedded in the cast-off material where they remain scattered until they are fully developed and then become free in the lumen of the tubule. This indicates that the sperms in that stage of development are attracted more by their own thrown-off material than by the rather scanty contents of the nurse cells, which are then very

much collapsed, owing to the large number of developing sperms which they have nourished. This migration of the sperm backward is probably nothing more than a chemotactic response to the food contained in the balls of cytoplasm.

XI. The Chromatoid Body.

The behavior of the chromatoid body in the horse bears a striking resemblance to the behavior of the chromatoid body described in *Pentatoma* (Wilson, '13). Dr. Wilson treats the subject at considerable length in his paper and therefore much of the detail concerning this body in the horse may safely be omitted. However, all the more essential features will be presented here since this is the first case among the vertebrates, according to my knowledge, where such an element has been studied in full detail. The reader is advised to familiarize himself with Professor Wilson's article in order to appreciate fully the surprising similarity existing in the behavior of the chromatoid body in such diverse classes of animals as the insects and the mammals.

In speaking of the chromatoid body Professor Wilson ('13) says in part, "As seen during the growth-period and the spermatocyte-divisions it is of rounded form, dense and homogeneous consistency, and after double staining with hæmatoxylin or safranin and light green is at every stage colored intensely blue-black or brilliant red, precisely like the chromosomes of the division-period or the chromosome-nucleoli of the growth-period. In the first spermatocyte-division it may lie anywhere in the cell, sometimes almost at the periphery, but is often close beside the chromosomes. In the latter case it usually lies in, on or near the spindle, lags behind the chromosomes during the anaphases, and in later stages is found near one pole, presenting an appearance remarkably like that of an accessory chromosome (Figs. 8–10). For such in fact I mistook it, even after the discovery that a similar body is often also seen near one pole in the *second* division (Figs. 22, 23); for I supposed this might be a case like that of *Ascaris megalocephala*, where, according to Edwards ('10) the *X*-chromosome may pass undivided to one pole in either the first or second division. The resemblance is indeed most deceptive; and these division-figures have often been exhibited to

other observers as "a remarkably clear demonstration of an accessory chromosome" without at first arousing the least suspicion of the hoax.

"The body in question is nevertheless neither an accessory nor any other kind of chromosome; though this did not become wholly certain until after a study of the entire spermatogenesis. It is in fact of protoplasmic origin, first appearing early in the growth-period outside the nucleus, whence it may be followed uninterruptedly through all the succeeding stages until it is finally cast out of the spermatozoön. Upon dissolution of the nuclear membrane it is left lying near the chromosomes, passes without division into one of the daughter-cells in each of the spermatocyte-divisions, and thus enters but one fourth of the spermatids."

In the horse the chromatoid body is of a spherical shape and also of a dense and homogeneous consistency, and stains exactly like the chromosomes of the division stages or the chromosome-nucleoli of the growth-period. It is invariably surrounded by a clear area. It makes its appearance in the stages immediately following synizesis and apparently attains its full size rather abruptly, for as a rule even in the earlier growing stages, if it is present at all, it possesses its full size, although in some cases it was found to be somewhat smaller (Fig. 13). Occasionally, in the earliest stages immediately following synizesis one or two very small bodies within clear vacuoles could be detected (Fig. 12). Two such small bodies are extremely rare and even the single minute bodies showing the very beginning of the chromatoid body are not numerous; however, it is quite certain that the body practically always originates as a single element. When the cells attain their maximum size the chromatoid body is invariably present and possesses its full size which makes it very conspicuous (Fig. 14).

The chromatoid body may be seen anywhere within the cytoplasm, either near the nucleus or far from it. Sometimes it appears to be in fairly close contact with the cell-wall (Fig. 14). When the nuclear wall disappears and the chromosomes come into full view, it may again be found anywhere in the cytoplasm. Later when the chromosomes arrange themselves for division

in the equatorial plate it most generally takes a position near them (Figs. 15 and 18), and when the spindle is formed, in a large majority of the cases, it takes a position in, on or near the spindle (Figs. 21–28) as is the case in Pentatoma (Wilson, '13). This, however, is not always the case, for occasionally it is far away from the spindle (Figs. 20 and 29).

It was in the primary spermatocyte division that the chromatoid body was first observed. It attracted my attention at the very first glance at the material under low power of the microscope and its constant appearance in this stage led me to suppose, at first, that it may be an accessory chromosome. Soon, however, the large, heart-shaped accessory was discovered and for some time I had the impression that this was the *X*-chromosome and the small spherical body the *Y*-chromosome. This temporary, erroneous impression was obtained through the peculiar fact that in about ninety per cent. of the cases the chromatoid body passes over to the half of the dividing cell opposite from that containing the large accessory, and in almost a hundred of the first mitotic stages examined not a single case was noticed in which the spherical body was on the same side of the equatorial plate with the accessory chromosome. Even when the first case in which both of the bodies were seen on their way to the same pole was observed, the matter was not taken very seriously. Later, however, when more such cases were seen, my suspicion was aroused and further observations convinced me that besides the supposed *y*-chromosome a body identical to it was present. And it was not until the entire history of the body could be traced from the growth-period to the casting-off of the mass of cytoplasm in the final stages of the developing spermatozoön, that I was absolutely certain that the suspicious looking element and the supposed *y*-chromosome were one and the same thing, namely, the chromatoid body, first described by Wilson in insects.

In exceptionally rare cases, one (Figs. 23 and 26) or two other small, deeply staining bodies within clear vacuoles occur in the cytoplasm (Fig. 27). However, in cases where such bodies do occur, there is no appreciable difference in the size of the chromatoid body and therefore it is difficult to determine whether such bodies are simply portions split off from the chromatoid

body, or whether they originate separately. In only three cases did I observe two bodies apparently of equal size and smaller than the profoundly constant chromatoid body (Fig. 54). Were such cases more numerous one might assume that such bodies are the components of the chromatoid body, but since such bodies are of such extremely rare occurrence no definite statement can be made in regard to them.

When the primary spermatocyte divides the chromatoid body is practically always found in only one of the resulting cells (Figs. 30–33) and in a large majority of the cases it is found in the cells which do not contain the accessory chromosome (Figs. 30, 31 and 33). This, however, is not universal, for in some cases at least, it is found in the same cell which contains the accessory (Figs. 32 and 35); and it has also been seen in the division stages of such a type of secondary spermatocyte (Figs. 38 and 39) as well as in the spermatid resulting from such a division (Fig. 41). In the anaphase of the secondary spermatocyte division the body is usually seen lagging on the spindle threads behind the masses of chromosomes (Figs. 39, 48, 49, 51 and 52); occasionally, however, it is seen at the pole (Fig. 51). After the division is complete the body usually lies far out in the cytoplasm (Figs. 41 and 57), and in rare cases only, is it seen in close contact with the nucleus. Figure 58 represents an extreme case of that nature, and it appears that such a condition is brought about when the chromatoid body bears a relation to the chromosome as is represented in Fig. 51. Sometimes two bodies (Fig. 60), though not always of the same size, appear in the spermatid.

In the late resting stages of the spermatid the body may again be found anywhere in the cytoplasm (Figs. 62–67), at times near the nucleus (Fig. 62). Sometimes it is found in close contact with the centrosome (Figs. 63 and 66) and in only rare cases it is found on the axial filament, giving the impression that it is fused or in close contact with the posterior centrosome (Figs. 72 and 76). Later, however, it leaves the filament and lies freely in the cytoplasm (Figs. 74, 75, 77, 86). In the final stages of the developing spermatozoön when the cytoplasmic mass is cast off, the chromatoid body when present is invariably thrown off with it (Figs. 82 and 86). It is certain that the chromatoid

body does not contribute in any visible way to the formation of the spermatozoön. The foregoing facts also indicate that great care must be exercised in interpreting the significance of bodies which appear like chromosomes, but really are something entirely different and no positive statements can be made regarding their meaning unless their entire history can be definitely traced.

It is very probable that a body similar to the chromatoid body in the horse also exists in the pig. In speaking of a small chromatin body which frequently occurs in the first spermatocyte division of the pig (Wodsedalek, '13), I make the following statement: "Occasionally a small chromatin body is present in this first spermatocyte division (Figs. 28, 31, 32, 35 and 37). Fig. 31 shows such a body passing to the same pole with the accessories, in advance of the other chromosomes. Fig. 32 represents an earlier stage of much the same thing. In Fig. 35 it can be seen passing to the opposite pole, and Fig. 37 represents an extremely rare case where two such bodies are present, one somewhat larger, passing to either pole, even in advance of the two accessory chromosomes. While the small body can be seen frequently, as a rule no such element can be detected, and while it may possibly be comparable to the small pair of chromosomes found so constantly in some of the Tracheata, my present data on its irregular occurrence and behavior do not permit a conclusion regarding its significance."

Further investigation regarding the body in question in the pig will be taken up presently. It might also be mentioned here that the chromatoid body is present in the germ-cell of the bull. A complete account of its behavior in that animal will be published later.

XII. Summary.

1. Thirty-seven chromosomes differing somewhat in size occur in the spermatogonia. One, the accessory, is distinctly larger than the others.

2. In the spermatogonial division the accessory divides a little in advance of the other chromosomes.

3. Nineteen chromosomes appear in the primary spermatocyte division, of which eighteen are evidently bivalent and the other is the accessory.

4. In the secondary spermatocyte division the heart-shaped accessory passes undivided to one pole in advance of the other chromosomes.

5. The primary spermatocyte division is evidently the reduction division, giving rise to two different types of secondary spermatocytes; one with the accessory and the other lacking it.

6. There is no resting stage following the first spermatocyte division.

7. A second pairing of the chromosomes takes place so that only one-fourth the original number of chromosomes appear for division in the secondary spermatocyte.

8. The accessory chromosome divides in the secondary spermatocyte division a little in advance of the other chromosomes the same as it does in the spermatogonia.

9. The one type of secondary spermatocyte, which contains the accessory, gives rise to two spermatids, each containing the accessory and nine bivalent chromosomes.

10. The other type of secondary spermatocyte, which lacks the accessory, gives rise to two spermatids, each containing only the nine bivalent chromosomes.

11. In terms of univalence, then, one type of spermatid receives eighteen chromosomes plus the accessory and the other type receives only the eighteen ordinary chromosomes.

12. In view of the foregoing facts, two different types of spermatozoa, equal in numbers, are produced in the horse; the one type contains in addition to the ordinary chromosomes the accessory, and is apparently the female determining spermatozoön.

13. Actual measurements of six hundred mature spermatozoa reveal the interesting fact that two distinct types of spermatozoa as regards size are produced, the one being much larger and presumably the one which bears the accessory chromosome.

14. The dimorphic condition among the spermatozoa of the horse lends additional support to the chromosome theory of sex determination.

15. The developing spermatozoa invariably cast off a mass of cytoplasm.

16. A chromatoid body, which simulates the appearance of a

y-element in the primary spermatocyte division stages, makes its appearance during the growth period and can be traced forward until it is finally thrown off with the ball of cytoplasm in the developing spermatozoön. It does not contribute in any visible way to the formation of the spermatozoön.

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EXPLANATION OF PLATES.

PLATE I.

(All of the drawings were made with the aid of a camera lucida, × 2,400.)

FIG. 1. Early spermatogonial cell showing a large triangular nucleolus and two small nucleoli, one of which is spherical. Other cells in the same stage often show many more nucleoli.

FIG. 2. Resting stage of a full grown spermatogonial cell showing the large triangular nucleolus and several small nucleoli, one of which is spherical and can frequently be detected.

FIG. 3. Prophase of a spermatogonial division in which the chromosomes are still rather indistinct.

FIGS. 4 AND 5. Late prophase of spermatogonial division showing thirty-six ordinary chromosomes and the large accessory which can easily be distinguished.

FIGS. 6 AND 7. Metaphase of division in a spermatogonium showing the accessory dividing in advance of the other chromosomes. In Fig. 6 the cell appears smaller and the chromosomes are more crowded.

FIGS. 8 AND 9. Early and late resting stages of a primary spermatocyte, respectively. Both show the large and the small nucleolus.

FIG. 10. Primary stage just before synizesis showing a mass of fine threads and the two nucleoli.

FIG. 11. Primary spermatocyte in synizesis showing the nucleoli in a characteristic position out of the mass of threads.

FIG. 12. Primary spermatocyte following synizesis and synapsis. The threads scatter about in the nucleus.

FIGS. 13 AND 14. Spireme stage of a primary spermatocyte showing increase in size of the cytoplasm, nucleus and the large nucleolus, and the beginning of the chromatoid body.

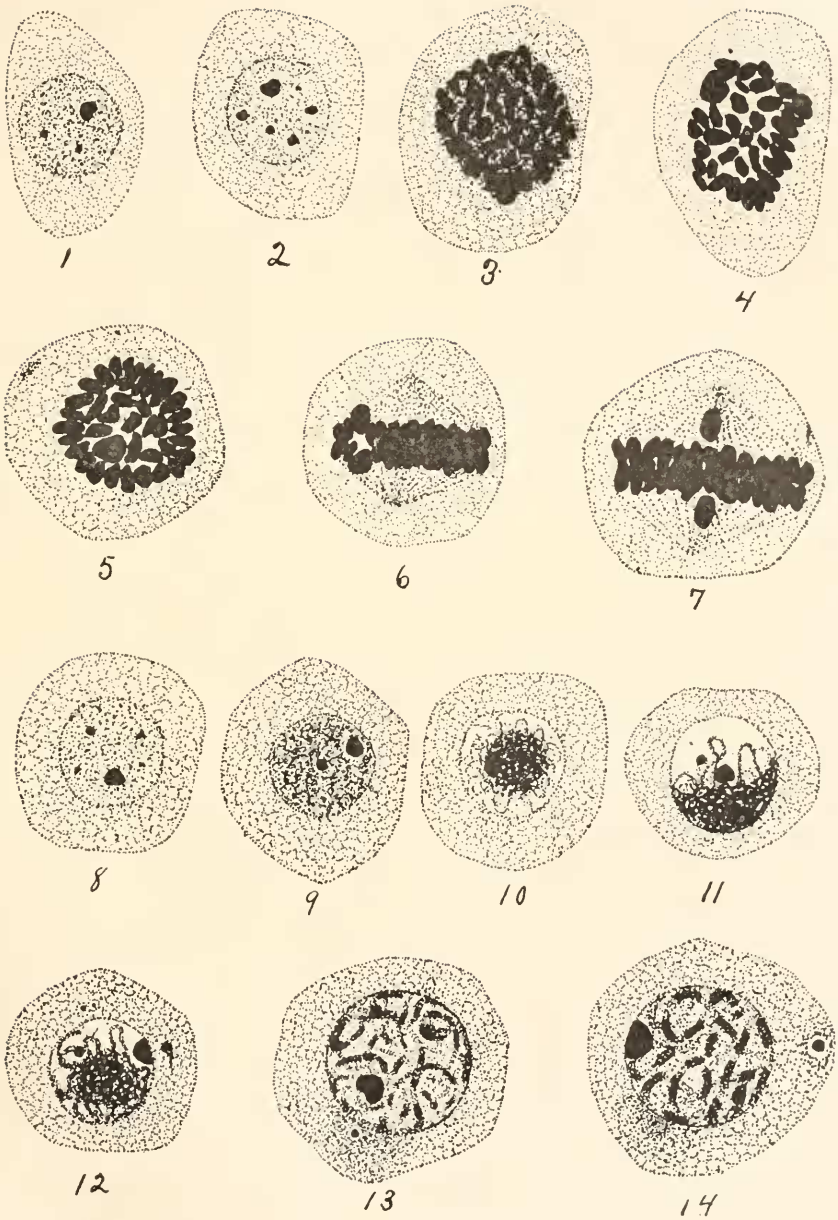

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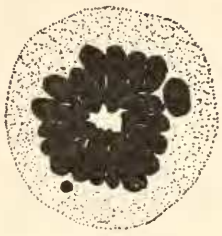
Plate II.

Figs. 15–18. Late prophases of primary spermatocytes showing eighteen large chromosomes, the accessory a little off to one side and the conspicuous chromatoid body anywhere in the cytoplasm. Fig. 16 shows a characteristic bunch of chromosomes in which a count is impossible.

Figs. 19–26. Metaphase of division in primary spermatocyte, showing the accessory chromosome in characteristic positions passing to the pole, and also the chromatoid body. Figs. 20 and 26 show the chromatoid body with the accessory on the same side of the equatorial plate. Figs. 23 and 26 show also an extra small body.



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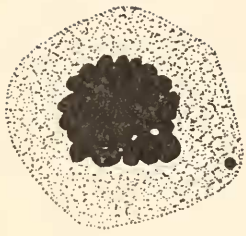

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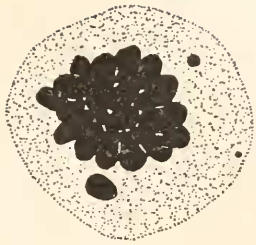

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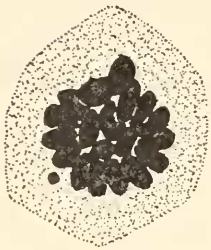

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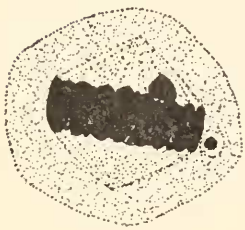

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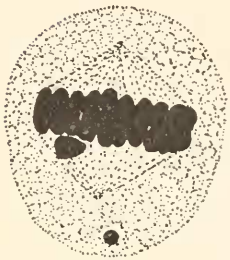

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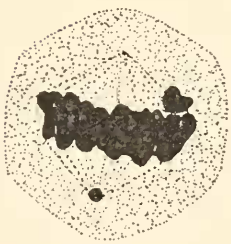

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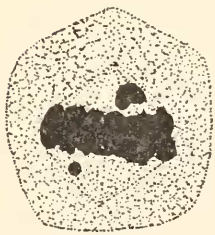

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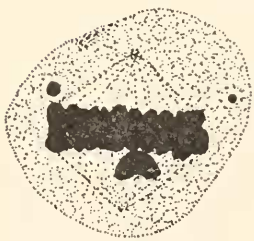

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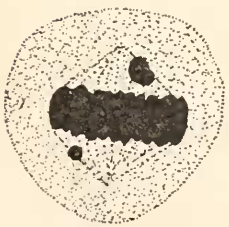

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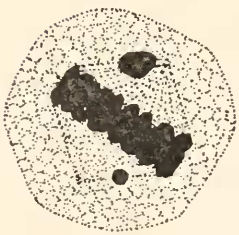

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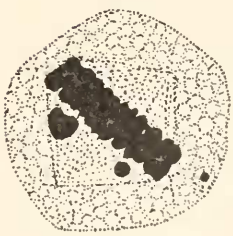

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PLATE III.

FIGS. 27, 28, AND 29. Metaphase of division in primary spermatocyte showing the accessory chromosome and the chromatoid body. Fig. 27 shows two other small and deeply stained bodies. Fig. 28 shows the accessory at one pole and the chromatoid body at the other. Fig. 29 shows the accessory at the pole, and the chromatoid body off the spindle and near the periphery of the cell.

FIGS. 30 and 31. Late anaphase of division in primary spermatocyte showing nine large chromosomes and the accessory at one pole, and nine large chromosomes and the chromatoid body at the other.

FIG. 32. Late anaphase of division in primary spermatocyte, showing nine chromosomes at one pole, and nine chromosomes, the accessory, and the chromatoid body at the other.

FIG. 33. Two resulting cells of a primary spermatocyte division, one containing the accessory chromosome and the other the chromatoid body.

FIGS. 34 and 35. Late prophase of division in a secondary spermatocyte which received the accessory chromosome. Cell represented in Fig. 35 also shows the chromatoid body.

FIGS. 36, 37, AND 38. Metaphase of division in the secondary spermatocyte showing the division of the accessory in advance of the other chromosomes. Fig. 38 also shows the chromatoid body near the periphery.

FIG. 39. Late anaphase of division in a secondary spermatocyte which received the accessory chromosome, nine apparently bivalent chromosomes and the large accessory can be seen at either pole and the chromatoid body is between the two masses of chromosomes.

FIGS. 40 AND 41. Spermatid showing nine bivalent chromosomes and the accessories. Fig. 41 also shows the chromatoid body.

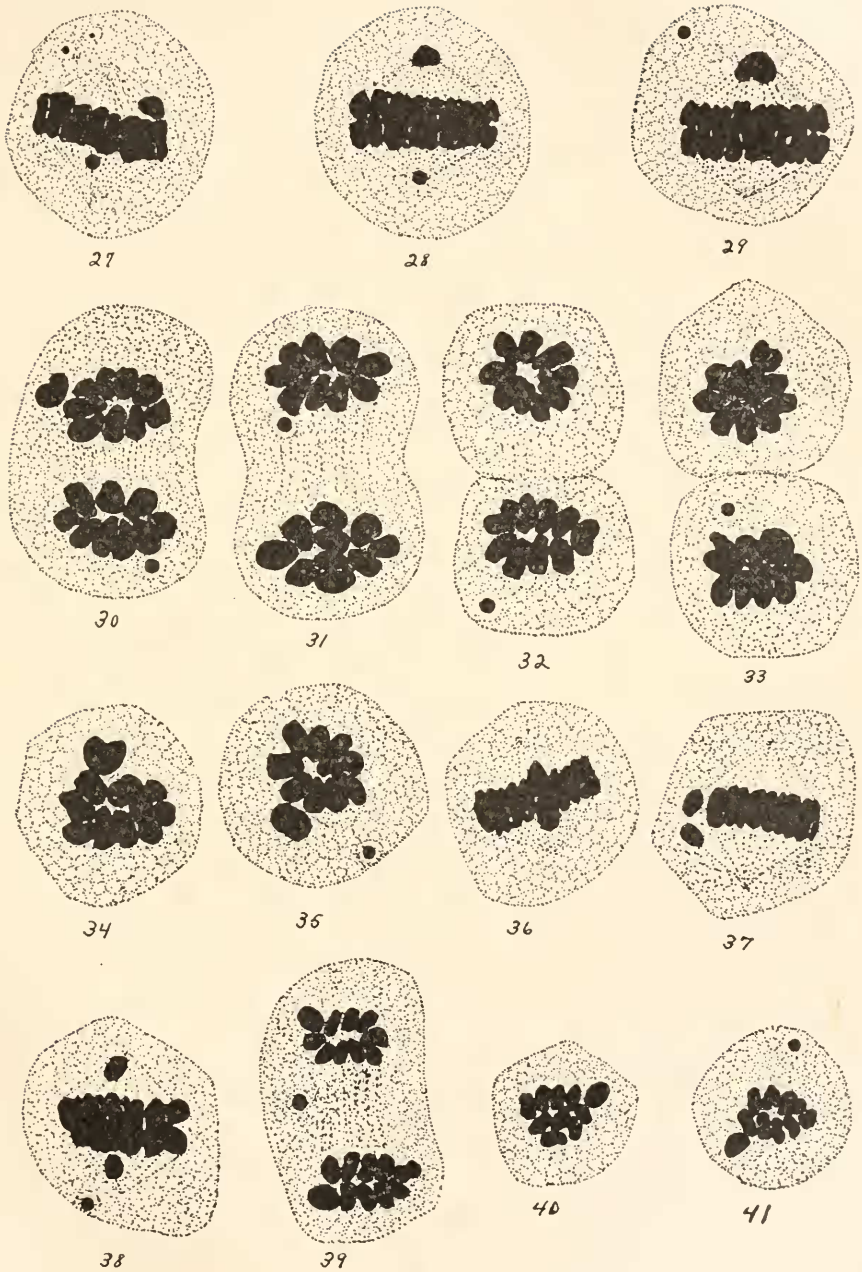

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Plate IV.

Figs. 42, 43, and 44. Late prophase of division in a secondary spermatocyte which did not receive the accessory chromosome, showing only the nine ordinary chromosomes. Figs. 42 and 43 show also the chromatoid body.

Figs. 45 and 46. Early metaphase of division in a secondary spermatocyte which did not receive the accessory chromosome. Fig. 45 shows the chromatoid body off the spindle.

Figs. 47–54. Late anaphase of division in secondary spermatocytes showing various positions of the chromatoid body when it is present. Fig. 47 shows also a small body in addition to the chromatoid body. The cell is one which did not receive the accessory chromosome. In the cell represented in Fig. 50 the chromatoid body was absent and in Fig. 54 two bodies may be seen.

Figs. 55 and 56. Spermatid showing nine bivalent chromosomes which is one of the resulting cells of the division of a secondary spermatocyte which did not receive the accessory chromosome. Fig. 55 shows the chromatoid body.

Fig. 57. Characteristic massing of the chromosomes just before the nuclear wall of the spermatid is formed.

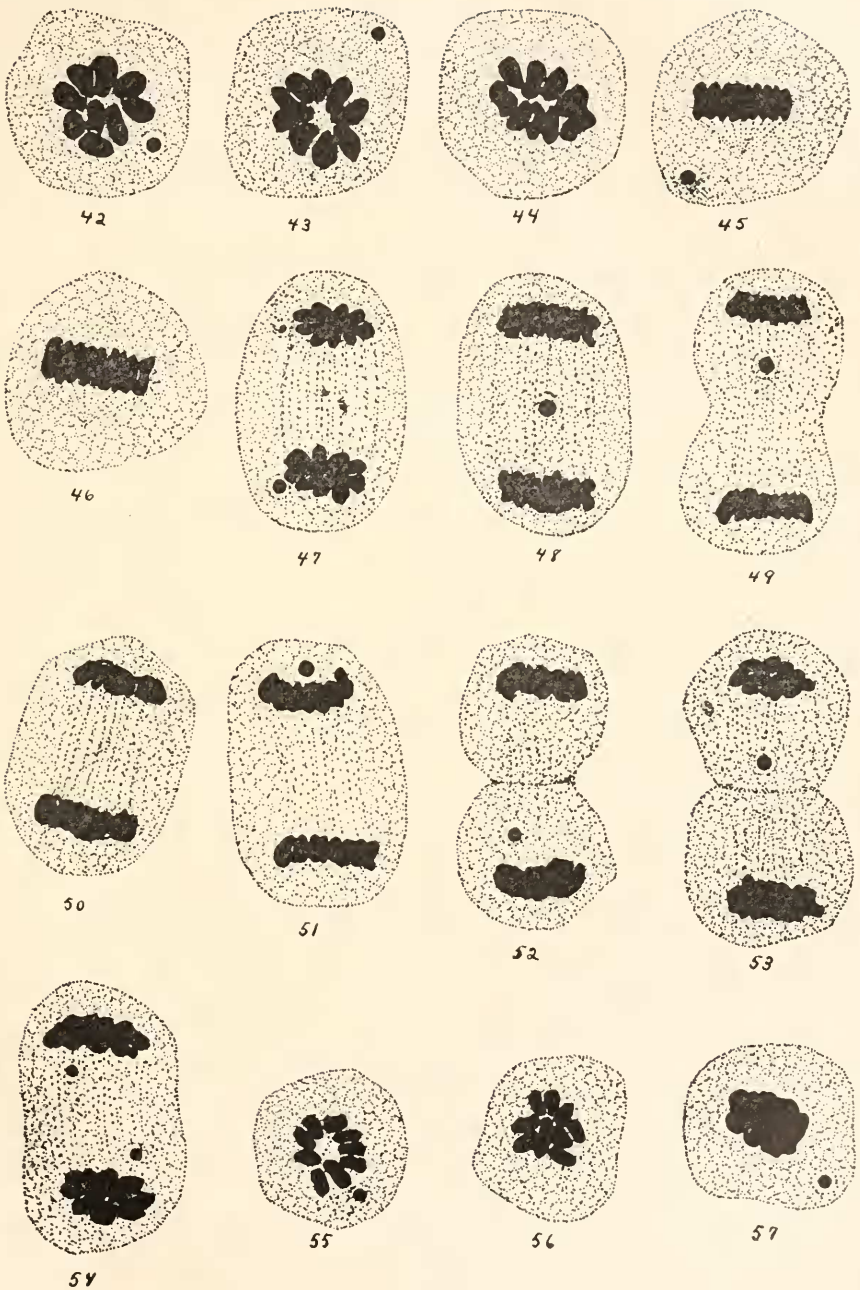

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PLATE V.

FIGS. 58–61. Early spermatids showing the characteristic structure of the nucleus, and the position of the chromatoid body. Fig. 60 shows two bodies of practically the same size as the chromatoid body.

FIGS. 62–67. Resting stage of the spermatid. Figs. 62–65 show the large nucleolus or the accessory chromosome. Fig. 62 shows the chromatoid body near the nucleus; Fig. 63 shows it very near the centrosome which is out of its sphere; Fig. 65 shows it at the periphery of the cell; Fig. 66 shows it near the centrosphere; and Fig. 67 shows it far from the centrosphere out of which the centrosome had just emerged.

FIGS. 68–73. Early stages of the developing spermatozoön. Fig. 68 shows the divided centrosome, the very beginning of the axial filament, and the acrosome which had migrated to the anterior end of the nucleus or sperm-head; Fig. 69 shows the posterior centrosome passing down the axial filament, and the chromatoid body far down in the cytoplasm away from the filament; Fig. 70 shows the same thing except that the chromatoid body is not present; Fig. 71 shows the chromatoid body near the posterior centrosome; Fig. 72 shows what apparently is the fusion of the chromatoid body with the posterior centrosome; and in Fig. 73 the chromatoid body is absent and the posterior centrosome is far down the axial filament and so small that it can scarcely be detected.

FIGS. 74 AND 75. Later stages of the developing spermatozoön showing the chromatoid body in the cytoplasm at the posterior end. Fig. 74 shows the posterior centrosome still on the filament, while Fig. 75 shows that it had been sloughed off.

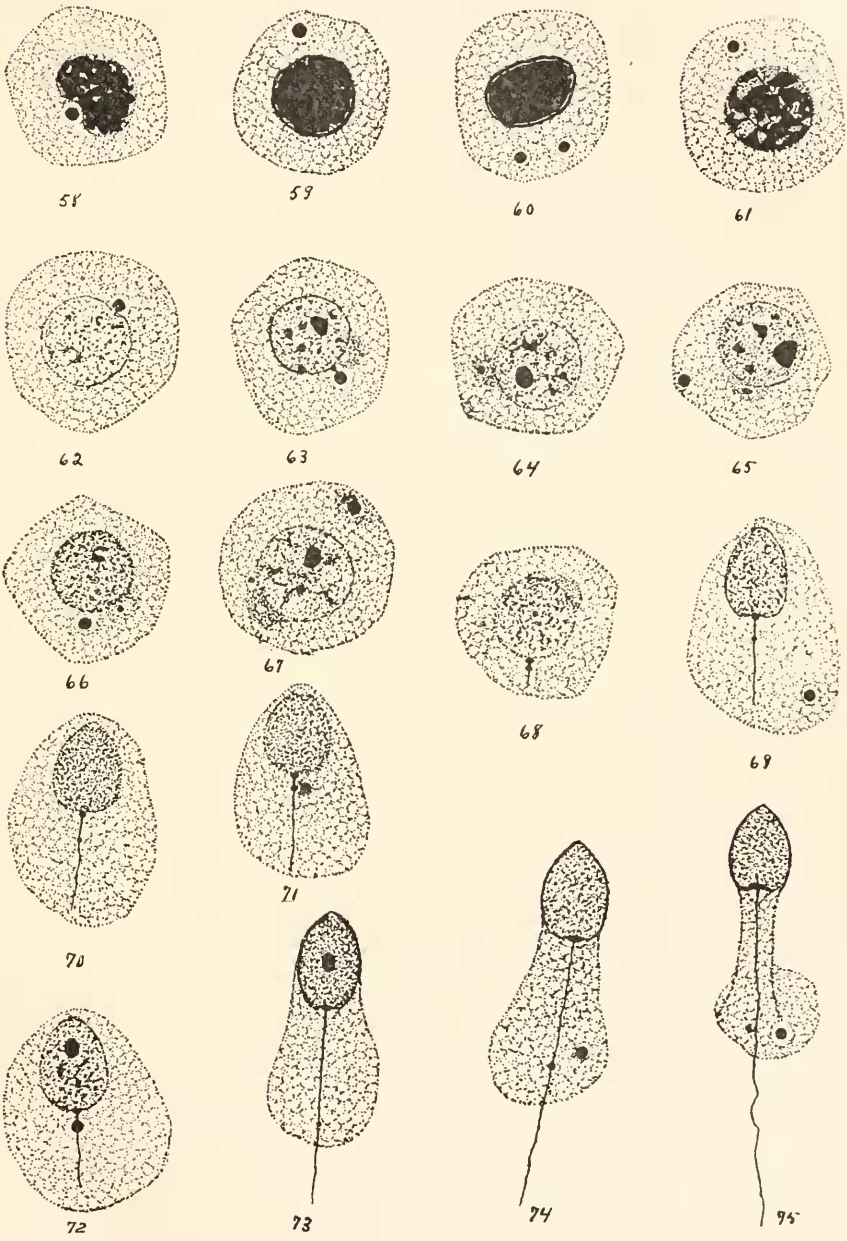

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PLATE VI.

FIGS. 76–79. Later stages of the developing spermatozoön. Fig. 76 shows the chromatoid body far down on the axial filament; Fig. 77 shows the chromatoid body very close to the posterior centrosome; Fig. 78 shows the sloughed-off centrosome, but the chromatoid body is absent; and in Fig. 79 both of these bodies are lacking

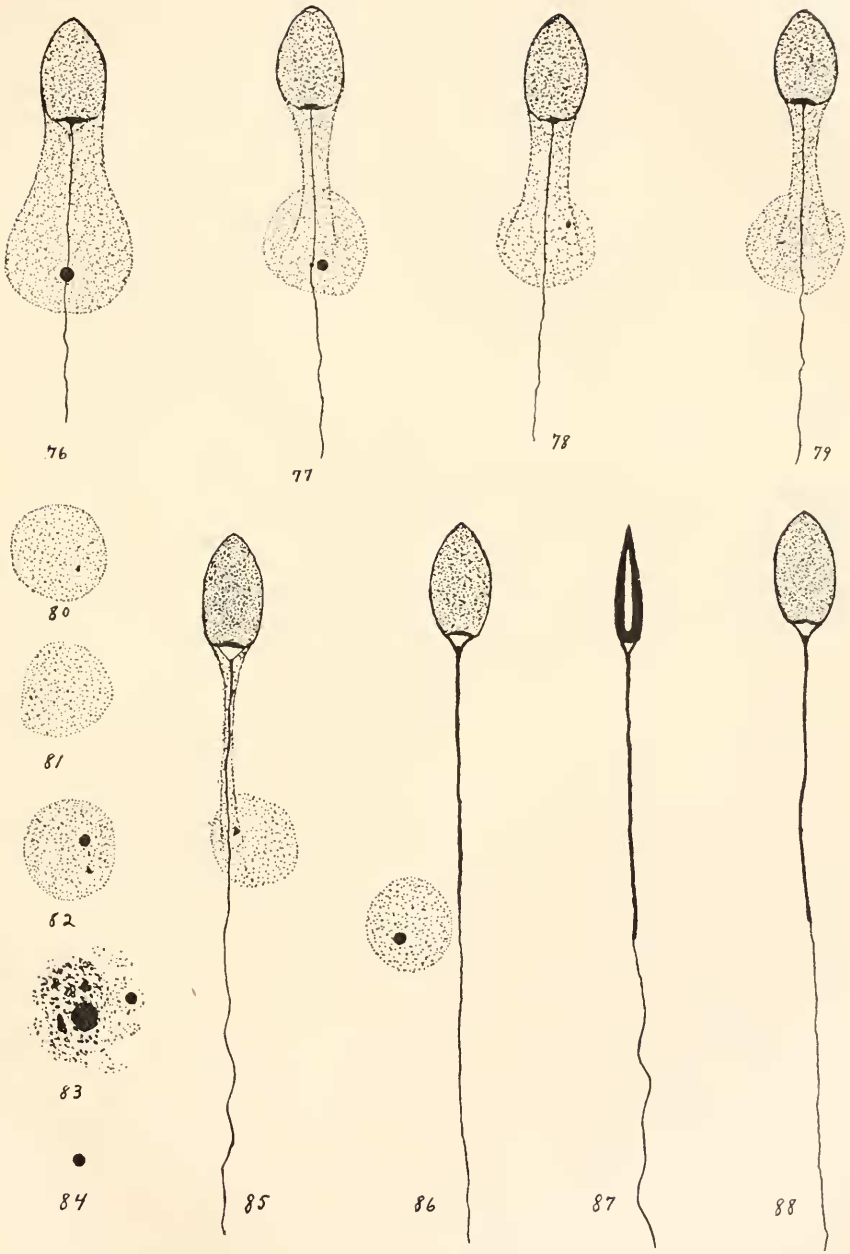
FIGS. 80–83. Cast-off balls of cytoplasm. Fig. 80 shows a small body which apparently is the sloughed-off centrosome; Fig. 81 shows neither the centrosome nor the chromatoid body; Fig. 82 shows both bodies; and Fig. 83 represents the ball of cytoplasm in the process of degeneration.

FIG. 84. A deeply staining body occasionally found in the lumen of the tubule and probably the same thing as the chromatoid body.

FIGS. 85 AND 86. Final stages in the developing spermatozoön. Fig. 85 shows that the cytoplasmic ball is about to be thrown off; and Fig. 86 shows the cytoplasmic mass together with the chromatoid body completely separated from the spermatozoön.

FIG. 87. Side view of a mature spermatozoön.

FIG. 88. A mature spermatozoön.



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AN EXPERIMENTAL STUDY OF THE AUDITORY POWERS OF THE GIANT SILKWORM MOTHS (SATURNIIDÆ)

C. H. TURNER,

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This is a companion paper to the "Auditory Powers of the Catocala Moths" by C. H. Turner and Ernst Schwarz. The latter paper embodies the results of a field study and this epitomizes a laboratory investigation. The habits of resting quietly upon a tree trunk and of flying, when disturbed, to a nearby tree renders the Catocalæ excellent material for field study; the fasting habits of the Saturniidæ render them equally good material for laboratory work. The paper on the Catocala moths contains both a historical resume and a bibliography; hence they are not needed in this contribution.

In these experiments the following moths were used: 79 specimens of *Samia cecropia* Linn., 104 of *Philosamia cynthia* Drury, 41 of *Callosamia promethea* Drury and 81 of *Telea polyphemus* Cramer. These insects were confined beneath wire dish covers. Each moth was numbered and one insect, in case of mated individuals one pair, was placed beneath a cover.

These experiments were conducted in an out-of-doors insectary the north wall of which is constructed almost entirely out of wire netting. The other three wooden walls are window-less and lined with shelves. These walls and the shelf-rests are supported by the ground. The wooden floor rests on the ground, but is not attached either to the walls or the shelf-rests; indeed, a space of from one to three feet separates the floor from the walls. Suspended from the ceiling by picture wire, there is a heavy swinging shelf. The subjects of these experiments were kept on these shelves. Since I always stood on the floor when sounding any of the instruments, it was impossible for the vibrations to reach the moths by any medium other than the air.

These experiments were conducted in the mornings between five and half past seven and in the afternoons between three and seven. On Saturdays and Sundays experiments were sometimes conducted all day long.

For producing stimuli the following instruments were used: an adjustible organ pipe, with a range for all notes of two octaves and for one note of three; an adjustable pitch pipe, and an Edelmann's Galton whistle. Such moths as responded did so by moving the wings as though about to fly. In the early experiments, before I had many moths on hand, each moth was tested with all of these instruments; because I hoped to determine the upper and lower threshold of hearing for each specimen. Later on, partly because I became convinced that there are theoretical

Table I.

Number: 3–VI–14: 1. *Specimen: Callosamia promethea*, female.

Place: Confined, under a wire dish cover, on the swinging shelf.

Method: At each trial the instrument was sounded five times at intervals of a minute and records made of the moth's behavior.

Trials.	Date.	Time.	Stimulus.	Vibrations Per Second.	Temperature.	Tests.					Remarks.
						1	2	3	4	5	
1	3–VI	6:30	P.P.	680	71	*	*	*	*	*	Response vigorous.
2	4–VI	6:00	P.P.	680	78	*	*	*	*	*	Response vigorous.
3	4–VI	6:10	G.W.	3,480	78	—	—	—	—	—	
4	4–VI	6:15	O.P.	512	78	*	*	*	*	*	Response vigorous.
5	4–VI	6:20	O.P.	256	78	*	*	*	*	*	Response slight.
6	4–VI	6:25	O.P.	128	78	*	*	*	*	*	
7	4–VI	6:30	O.P.	64	78	*	*	*	*	*	
8	6–VI	10:05	P.P.	680	86	—	—	—	—	—	Whistle held in rear.
9	6–VI	10:10	O.P.	512	86	—	—	—	—	—	Whistle held in rear.
10	6–VI	10:15	O.P.	256	86	—	—	—	—	—	Whistle held in rear.
11	6–VI	10:20	O.P.	128	86	—	—	—	—	—	Whistle held in rear.
12	6–VI	10:25	O.P.	64	86	—	—	—	—	—	Whistle held in rear.
13	6–VI	10:30	P.P.	680	86	*	—	—	—	—	Whistle held in front.
14	6–VI	10:35	O.P.	256	86	*	*	*	*	*	Whistle held in front.
15	6–VI	15:00	P.P.	680	96	*	*	*	—	—	Whistle held in front.
16	6–VI	15:10	O.P.	256	96	—	*	—	*	*	Whistle held in rear.
17	6–VI	15:20	O.P.	64	96	—	—	—	—	—	
18	6–VI	15:30	O.P.	256	96	*	*	*	*	*	

Explanation of abbreviations; G.W. means Galton whistle; O.P., organ pipe; P.P., pitch pipe; in the second column, the roman numerals stand for months and the Arabic for days; in the third column, the hours are numbered from 1 to 24, beginning at 1 A.M.

reasons why the thresholds cannot be accurately determined by this method and partly on account of practical difficulties, I confined my experiments to a few notes of the middle range. When I remind you that I often had on hand from fifty to seventy-five moths, you will readily see that it was impossible to test each moth, each time, with the entire range of pitches.

The results of these investigations were recorded upon blanks that were prepared especially for this work. A portion of one of those blanks is reproduced in the preceding table.

After the work on all of the moths had been completed, the contents of these blanks were condensed into the following tables.

TABLE II.

REACTIONS OF GIANT SILK-WORM MOTHS TO SOUNDS.

Name of the Specimen.	Number of Individ.	Number of Trials.	Per Cent. of Responses.										
			0	1 to 9.	10 to 19.	20 to 29.	30 to 39.	40 to 49.	50 to 59.	60 to 69.	70 to 79.	80 to 89.	90 to 100.
Samia cecropia													
Males	38	380	1	0	0	0	1	0	1	2	0	0	33
Females	41	615	0	0	0	1	0	1	5	0	1	1	32
Total	79	995	1	0	0	1	1	1	6	2	1	1	65
Philosamia cynthia													
Males	50	950	19	0	0	2	4	4	9	3	1	1	7
Females	54	875	10	0	1	3	0	1	11	4	4	1	19
Total	104	1,825	29	0	1	5	4	5	20	7	5	2	26
Callosamia promethea													
Males	23	380	4	0	0	0	3	0	5	0	0	1	10
Females	18	495	1	0	0	0	1	0	1	2	5	2	6
Total	41	875	5	0	0	0	4	0	6	2	5	3	16
Telea polyphemus[1]													
Males	39	950	36	0	0	1	0	0	0	0	0	0	2
Females	39	950	39	0	0	0	0	0	0	0	0	0	0
Total	78	1,900	75	0	0	1	0	0	0	0	0	0	2

[1] The above table does not record the three specimens of *T. polyphemus*, which were used in the special tests recorded on pages 333–334.

TABLE III.

RESPONSES OF *Samia cecropia* TO SOUND.

Instrument.	Pitch Vibra. per Second.	Individuals Participating.			Number of Trials.			Per Cent. of Response.		
		Males.	Females.	Total.	Males.	Females.	Total.	Males.	Females.	Total.
O.P.	64	1	4	5	5	35	40	100	100	100
O.P.	128	2	1	3	10	5	15	50	100	67
O.P.	256	6	13	19	25	75	100	100	100	100
O.P.	512	1	2	3	5	15	20	100	100	100
P.P.	680	26	34	60	310	360	670	94	89	91
P.P.	870	2	2	4	15	10	25	33	0	20
G.W.	3,480	11	19	30	60	100	160	100	70	81
G.W.	4,645	0	1	1	0	5	5		100	100
G.W.	6,200	1	0	1	10	0	10	50		50
G.W.	6,960	0	1	1	0	5	5		100	100
G.W.	9,290	1	0	1	5	0	5	100		100

Explanation of abbreviations used in above table: O.P., organ pipe; P.P., pitch pipe; G.W., Galton whistle (Edlemann's).

TABLE IV.

EFFECT OF AGE ON THE RESPONSES OF *S. cecropia* TO SOUND.

Age in Days.	Individuals Participating.			Number of Trials.			Per Cent. of Responses.		
	Males.	Females.	Total.	Males.	Females.	Total.	Males.	Females.	Total.
0–1	26	23	49	205	200	405	80	80	80
1–2	7	10	17	60	55	115	100	82	91
2–3	5	11	16	30	65	95	100	54	72
3–4	2	12	17	10	120	130	100	100	100
4–5	4	4	8	20	25	45	100	100	100
5–6	2	3	5	10	20	30	100	75	83
6–7	4	3	7	25	30	55	100	83	91
7–8	1	2	3	15	20	35	100	100	100
8–9	1	2	3	10	15	25	100	100	100
9–10	2	4	6	25	30	55	100	100	100
10–11	0	3	3	0	15	15		100	100

TABLE V.

EFFECT OF TEMPERATURE ON THE RESPONSES OF *S. cecropia* TO SOUNDS.

Temperature in F. Degrees.	Individuals Participating.			Number of Trials.			Per Cent. of Responses.		
	Males.	Females.	Total.	Males.	Females.	Total.	Males.	Females.	Total.
50–59	11	6	17	130	70	200	83	53	70
60–69	9	14	23	130	110	240	74	87	80
70–79	4	16	20	40	170	210	100	94	95
80–89	18	27	45	155	235	290	97	96	96
90–99	0	4	4	0	35	35		100	100

TABLE VI.

EFFECT OF MATING ON THE RESPONSES OF *S. cecropia* TO SOUND.

	Number of Individuals.	Number of Trials.	Per Cent. of Responses.
Males:			
Unmated.....	31	320	88
Mated	7	120	97
Total.....	38	440	90
Females:			
Unmated.....	36	520	86
Mated.....	5	55	73
Total.....	41	575	85
Grand total.....	79	1,015	88

TABLE VII.

RESPONSES OF *Philosamia cynthia* TO SOUND.

Temperature in F. Degrees.	Individuals Participating.			Number of Trials.			Per Cent. of Responses.		
	Males.	Females.	Total.	Males.	Females.	Total.	Males.	Females.	Total.
60– 69	25	23	48	145	120	265	31	42	33
70– 79	43	43	86	410	420	830	36	60	48
80– 89	23	34	57	245	300	545	33	67	51
90– 99	6	11	17	130	65	195	58	77	67
100–109	0	8	8	0	40	40		88	88

TABLE VIII.

EFFECTS OF MATING ON THE RESPONSES OF *Philosamia cynthia* TO SOUND.

	Number of Individuals.	Number of Trials.	Per Cent. of Responses.
Males:			
Unmated.....	47	880	38
Mated.....	8	65	31
Total.....	55	945	36
Females:			
Unmated.....	50	835	63
Mated.....	8	90	56
Total.....	58	925	61

Table IX.

Effect of Age on the Responses of *Philosamia cynthia* to Sound.

Age in Days.	Individuals Participating.			Number of Trials.			Per Cent. of Responses.		
	Males.	Females.	Total.	Males.	Females.	Total.	Males.	Females.	Total.
0–1	45	51	96	645	400	1,045	34	58	43
1–2	26	19	45	290	160	450	49	68	57
2–3	12	18	30	160	365	525	38	73	53
3–4	19	19	38	145	105	250	32	58	42
4–5	6	12	18	45	70	115	32	64	48
5–6	1	6	7	5	30	35	100	83	85
6–7	1	1	2	5	5	10	0	100	50
7–8	2	4	6	10	20	30	0	50	34
9–10	0	2	2	0	10	10	0	50	50

Table X.

Effect of Temperature on the Responses of *Callosamia promethea* to Sound.

Temperature in F. Degrees.	Individuals Participating.			Number of Trials.			Per Cent. of Responses.		
	Males.	Females.	Total.	Males.	Females.	Total.	Males.	Females.	Total.
50–59	1	0	1	5	0	5	100		100
60–69	7	3	10	50	20	70	70	75	71
70–79	10	13	23	125	125	250	72	80	76
80–89	16	18	34	135	250	385	56	76	69
90–99	10	18	28	70	150	220	71	87	82
100–109	0	8	8	0	40	40		63	63

Table XI.

Effect of Mating on the Responses of *Callosamia promethea* to Sound.

	Number of Individuals.	Number of Trials.	Per Cent. of Responses.
Males:			
Unmated.....	19	375	63
Mated.....	3	55	82
Total.....	21	430	67
Females:			
Unmated.....	15	515	80
Mated.....	3	62	80
Total.....	18	575	80

A careful perusal of the tables I–XII. shows that *S. cecropia*, *P. cynthia* and *C. promethea*, respond to a long range of sound waves. Since precautions were taken to prevent vibrations reaching them through any medium other than air, it seems

Table XII.

Effect of Age on the Responses of *Callosamia promethea* to Sound.

Age in Days.	Individuals Participating.			Number of Trials.			Per Cent. of Responses		
	Males.	Females.	Total	Males.	Females.	Total.	Males.	Females.	Total.
0–1	17	13	30	155	105	260	74	76	75
1–0	14	14	28	125	135	260	68	89	79
2–3	11	13	24	60	125	185	50	92	78
3–4	7	10	17	35	75	110	30	75	58
4–5	2	8	10	10	105	115	100	57	61
5–6	1	6	7	5	45	50	0	56	50
6–7	0	1	1	0	5	5		100	100
7–8	0	1	1	0	5	5		100	100

reasonable to conclude that they hear. How about *Telea polyphemus?* Of the seventy-eight individuals whose behavior is recorded in Table II. only three made any responses whatever. Of these three, two gave over ninety per cent. of responses and the other less than thirty. Shall we conclude that *Telea polyphemus* is deaf and that these few responses were due to some factor overlooked by the investigator; or, shall we consider the responses made by all of these moths as expressions of emotion, and attribute the non-responsiveness of *polyphemus* to a sluggish temperament?

To one who has worked much with *Telea polyphemus*, this last suggestion is fascinating; for this moth is exceptionally unresponsive to all ordinary stimuli. The opposite sex is about the only thing that arouses much activity. There is another possibility. *Telea polyphemus* is not a very conspicuous object; indeed, in certain situations, it might be considered protectively colored. It may be that correlated with this inconspicuous coloration is an instinct to remain rigidly immobile in the presence of all ordinary stimuli. To test the matter the following experiments were conducted.

A freshly emerged *Telea polyphemus*, the wings of which had become thoroughly dry, was tested with an organ pipe set to produce 256 vibrations per second. As was to be expected, there was no visible response. The organ pipe was then sounded five times in rapid succession. Immediately thereafter, the insect was roughly handled for a few minutes. It was tossed

about, gently squeezed and thrown upon its back. This was repeated over and over again, sometimes in one order and sometimes in another. After the moth had quieted down, the pipe was sounded five times in rapid succession. Each time the pipe was sounded, the moth waved its wings vigorously. At intervals of two hours, this experiment was repeated from early morning until dark. Invariably the moth responded in the same manner. On the following day the experiment was continued with the same moth. The result was always the same.

About a week later, similar experiments were conducted with two other specimens of the same moth. These, like the one used above, were females. With two exceptions, the results were identical. The exceptions were as follows: (1) one of the moths instead of moving its wings vigorously moved them slowly; the other two moths moved their wings so vigorously that they were lifted off of the support; in this case the body remained on the support, although the wings moved each time the whistle blew; (2) on two occasions a moth that had been experimented upon several times, instead of waiting for the five tones that were produced after the handling, waved its wings vigorously to each of the five preliminary notes. Evidently *Telea polyphemus* can hear. These experiments induced in those moths a state of nervous excitability which caused them to respond to the sounds produced.

Conclusions.

1. It seems certain that all four of the species of giant silkworm moths investigated can hear. Three of the species respond readily to a large range of sounds. The third, *Telea polyphemus*, normally does not respond to sounds; unless remaining as immobile as possible be considered a response. By experimentally causing the moth to associate some disagreeable experience with certain sounds, it can be induced to respond to those sounds.

2. There is much evidence that the responses of moths to stimuli are expressions of emotion. The fact that an insect does not respond to a sound is no sign that it does not hear it. The response depends upon whether or no the sound has a life significance.

A PRELIMINARY ACCOUNT OF SOME CYTOLOGICAL CHANGES ACCOMPANYING DESICCATION.

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The ability of certain rotifers, tardigrades and nematode worms to withstand periods of desiccation has been a subject of investigation for many biologists throughout a period of more than two hundred years. Beginning with von Leeuwenhoek in 1701 and extending to the present time, researches have been carried on at intervals, in the case of the Bdelloid rotifers, with the object of determining whether or not these animals can undergo a true desiccation. The results of the several authors have shown a striking variance, in fact in some cases the conclusions of one worker have been directly opposed to those of some other who used the same species of animal in his experiments. The latest publication upon this subject is that of Jacobs ('09) who worked on the Bdelloid rotifer, *Philodina roseola*. He concludes, after prolonged experimentation and as a result of chemical and physical tests as well as by other indirect methods, that this animal undergoes a true desiccation; that at all times the cuticle is freely permeable to water and gases and that no evidence of a waterproof cyst can be found. He notes further that desiccation is usually followed by a period of reproductive activity. The foregoing conclusions together with others not bearing directly upon the subject of this paper have been confirmed during the course of this study.

Jacobs, while conducting his investigation in a very thorough manner, made no attempt to determine, from a histological or cytological point of view, the condition of the tissues of the desiccated animals as compared with those in the normal individual. To the knowledge of the author no comparison of this sort has been attempted by any investigator up to the present time. At the suggestion of Dr. E. G. Conklin, I have undertaken an inquiry into this last question and present here a brief account

of the results obtained to date. A more detailed account based upon further work will appear in a future publication.

The anatomy of the Philodinidæ has been described by Janson (1893). The most conspicuous organs in the living animal are those of the reproductive and the alimentary systems. The latter begins with the buccal cavity at the base of the trochal organs. This narrows down to a short tube leading to the mastax. The mastax, with the digestive glands surrounding it, is followed by the thin-walled esophagus which leads into the thick-walled stomach. The posterior end of the latter is continued by the "*blasendarm*" which leads to the cloaca and thence to the anus. The reproductive organs consist of two more or less spindle-shaped bodies lying on either side of the stomach. These are the ovaries and the vitellaria and they may at all times be recognized by their prominent nuclei. The nephridia with their flame cells are easily observed in the living animal. In the foot are found the glands which secrete a substance which enables the animal to adhere to different objects. The head region contains several large coronal cells with large nuclei.

A cross section of a normal extended *P. roseola* through the mid-body region is shown in Fig. 1. The vitellaria have enormous nuclei, consisting of a central karyosome surrounded by a clear homogeneous area and peripherally by a distinct nuclear membrane. This is the "nucleolar nucleus" of Carnoy and this type of nucleus is characteristic of the greater part of the cells of the animal. The cytoplasm of the vitellarium, which is syncytial in nature, is made up of granules of varying sizes and these granules appear in different patterns particularly when a variety of fixatives are used; it has quite an affinity for nuclear stains as is usual with yolk structures. The cytoplasm of the ovary does not differ essentially from that of the vitellarium. Ovary and vitellarium are each surrounded by a thin membrane. Figs. 1 and 4 show characteristic sections through normal reproductive glands.

The cytological structure of the stomach of the Philodinidæ has been described by Zelinka ('86) in the case of *Callidina symbiotica*, by Janson ('93), and more recently by de Beauchamp ('09), in the case of *Callidina socialis*. The lumen, the position

of which in the stomach tissue is not constant, is lined with a heavy ciliated cuticula. Just beneath the cuticula are found longitudinal muscle fibers arranged at regular intervals. The part of the stomach outside the thin layer of muscle fibers is syncitial in nature. With the iron-hæmatoxylin-eosin-lichtgrün stain of de Beauchamp three elements may be distinguished, (1) nuclei having, in general, the same structure as those described for the vitellarium; (2) densely staining granules of great size, not surrounded by a clear area or membrane. These are probably aggregations of food material. (3) Vacuoles of greater or less size which stain with lichtgrün. These last are probably globules of excretory material as de Beauchamp has pointed out. In the latter's description of the stomach of *Callidina socialis* he says that the stomach is not surrounded by an "individualized membrane" but only by a layer of protoplasm which projects at the periphery. In *P. roseola*, as far as I have been able to determine, a true membrane is present (Fig. 1).

The skin of *P. roseola* has practically the same structure as that of other rotifers. It consists of two layers, cuticula and hypodermis. The former is the more densely staining layer and is composed of fine granules closely packed together; the latter is a finely reticulated plasma layer in which cell boundaries cannot be distinguished and in which nuclei are found scattered at irregular intervals. The skin is pliable and may be readily folded at any point. It is difficult to obtain sections in which one or more of these folds do not appear.

The brain of *P. roseola* is of an elongated triangular shape and lies in front of and slightly above the mastax. Zelinka ('88), in the case of *Discopus synaptæ*, has figured the brain as a syncitium in which the nuclei are closely packed together about the periphery, while in the central part is found the "*punkstubstanz*," a finely granular portion without nuclei. In the greater number of cases I have been able to distinguish definite cell boundaries in the case of the cells forming the peripheral layer of the brain of *P. roseola*. The nuclei of these cells are uniformly circular in section and contain a small amount of chromatin scattered in irregular masses through a homogeneous nuclear plasm. The cytoplasm is homogeneous and has the appearance of a colorless

fluid. The "*punktsubstanz*" lies approximately at the center of the organ and is granular in structure.

In the fully extended living *Philodina* the ciliated or trochal discs are prominent at the anterior end of the body. The cilia upon these discs by their successive beating give the effect of a revolving wheel. When the animal is disturbed the discs are folded and retracted into the pharyngeal region where they may be observed as oval patches. The alimentary canal is also ciliated throughout almost its entire length.

Of the glandular structures, other than those employed for reproduction, the slime glands of the foot are perhaps most easily seen. These consist of rows of cells whose cytoplasm is alveolar or finely reticular. The nuclei are large and may sometimes be seen in the living animal. The digestive glands in the region of the mastax are similar to the foot glands in structure and staining qualities.

The changes in cell organization which accompany the process of desiccation are fairly uniform in result for all the tissues. Although slight variations have been observed, these are differences of degree and not of kind. Since the cellular elements are larger and hence more easy to observe in the vitellarium, this organ will be considered first.

In a section of the vitellarium of a desiccated *P. roseola* the most noticeable difference from the conditions which are present in the normal tissues are seen in the nucleus. Normally, as was stated before, the nuclear membrane, though definite, is not very thick. Just within the membrane is a ring of homogeneous ground substance or nuclear sap. In the center of the nucleus is found the large, densely staining karyosome. In the dried animal these conditions are exactly reversed. The karyosome may disappear entirely but if this extreme condition does not come about, the structure which remains in the position of the karyosome is similar neither in shape nor in staining qualities to the original element. In extreme cases the central area of the nucleus in the dried organ has exactly the same appearance as the clear area of the normal nucleus. The nuclear membrane becomes heavy and has the appearance of a thick ring (Fig. 5). In most cases it appears to be composed of fine granules closely

packed together. Under conditions mentioned hereafter this granular appearance may give place to a dense homogeneous black ring (iron-hæmatoxylin preparations) staining exactly like the normal karyosome. The changes in the cytoplasm, though distinct, are much less marked than the nuclear changes. With the withdrawal of water the cytoplasm increases in density and loses the regular arrangement of its particles which is characteristic of the normal vitellarium (Fig. 4). The yolk granules become arranged irregularly or in small closely packed groups as in Fig. 5. The drying process causes a loss of staining power in the tissue.

The shrinkage of the cytoplasmic portion of the tissues is well demonstrated in the case of the hypodermis. Fig. 1 show as section of this layer of the skin as it appears in the animal living under normal conditions. In a section through the dried animal (Fig. 2) it will be noticed that the hypodermal layer has shrunken markedly, approaching its normal thickness only in those places where the nuclei are located. The nuclei apparently do not diminish in size and they cause a protuberance in the dried hypodermis wherever they are found. The nuclear material is redistributed in the same manner as was described for the vitellarium.

This arrangement of the nuclear elements is found in practically all the tissues of the dried animals. A detailed description of the changes in the other organs would be, for the most part, mere repetition.

As was mentioned above, the cilia in *P. roseola* are well developed, both in the head region and in the digestive tract. It would seem that a fiber of such delicate texture as that of a cilium would not long survive the effects of a removal of moisture. Such, however, is not the case. Not only do the trochal cilia escape serious injury by the desiccation process but the same is also true of those in the digestive canal. Fig. 2 shows a section cut through the infolded trochal discs of a dried animal. There is no sign of any fusion or other abnormal condition of these elements. Each cilium preserves its identity apparently as well as would those of an animal living in a natural environment.

The changes in cell structure attending recovery from desicca-

tion are almost the exact opposite of those just described. In cases where the karyosome has entirely disappeared it begins to form again in its proper position a short time after water is added to the dried animals. The thickened nuclear membrane described above shows a greater affinity for stains at this stage and gradually assumes its normal thickness. Cytoplasmic changes are quite noticeable at this time. In the vitellarium (Fig. 6) it is frequently noticed that the material surrounding the nucleus is aggregated into strands or other irregular patterns. This would seem to indicate that the cytoplasm is more freely permeable to water in certain regions than in others and that the stage represented in Fig. 7 shows a step in the gradual redistribution of extranuclear substance attending recovery from desiccation. In the case of the other organs, as before, the process of recovery is very similar. The elements are much smaller in some cases and hence more difficult to observe but the mechanism as well as the result seems to be the same.

It has been suggested to the author that the cytoplasmic and the nuclear changes taking place in dry seeds might be analogous to the ones in the rotifers just described. With this in mind, sections of the embryo of the common Indian corn, *Zea mais*, were cut, (1) at the time the seeds were fully ripened but had not become entirely dried; (2) after the seeds were thoroughly dried; and (3) after the seeds were well germinated. A section of a typical procambium cell from each of these stages is shown herewith. Fig. 14 shows a cell from a germinating embryo. It will be noticed that the cytoplasm contains many spaces filled with cell sap. The nucleus has a ring of chromatic material just within the nuclear membrane. The nucleolus is vacuolated and does not stain in the same manner as the chromatic ring at the periphery of the nucleus. The nucleolus is surrounded by a clear area which probably consists of fluid material. Fig. 15 shows the conditions which exist when the embryo is partially dried. The chromatic ring thickens, diminishing the space between it and the nucleolus. The latter becomes more compact and the vacuoles disappear. An extreme case of drying is shown in Fig. 16. The cytoplasmic granules are closely and regularly packed together. The clear space in the nucleus has disappeared

and the substance of the nucleolus has apparently diffused throughout the nuclear area.

The changes described for the drying corn cells in the last paragraph are at first sight remarkably like those occurring in the rotifer during desiccation. In both rotifer and corn the nucleus contains a nucleolus surrounded by a clear space, while around the two is a chromatic membrane of varying thickness. When water is removed the clear space around the nucleolus disappears and comes into existence again only upon the addition of water. The substance of the nucleolus in both cases diffuses toward the periphery of the nucleus leaving a more or less clear space in the center of the same. In the cytoplasm also there is a parallel between the behavior of the cells of the two forms. Loss of water is attended by shrinkage and a consequent increase in density. The cytoplasmic materials tend to gather in small lumps which remain closely packed together until moisture is again applied.

Whether the seemingly similar processes in these representatives of the plant and animal kingdom are indeed analogous can be determined only after further study.

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Description of Plate I.

Fig. 1. Cross section through the mid-body region of a normal expanded *Philodina roseola*. Leitz compensating ocular 4, obj. 2mm.

Fig. 2. Section of a rotifer kept for eighteen days previous to fixation in an evacuated calcium chloride desiccator. Leitz oc. 4, obj. 2mm.

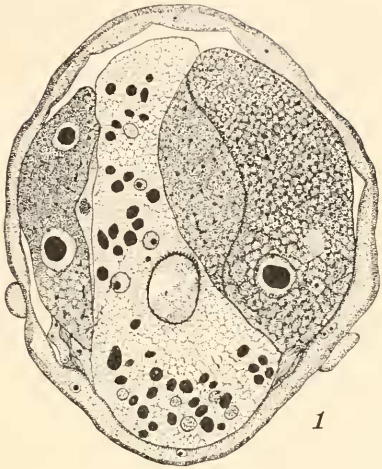
Fig. 3. Section through a normal animal, not expanded. Leitz oc. 4, obj. 2mm.

Fig. 4. Section through the vitellarium of a normal animal. Leitz oc. 12, obj. 2mm.

Fig. 5. Section of the vitellarium of an animal dried in an evacuated desiccator for fourteen days previous to fixation. Leitz oc. 8, obj. 2mm.

Fig. 6. Longitudinal section of an animal recovering from desiccation. The rotifer was kept in an evacuated desiccator for fifteen days, then placed in water for an hour and fifteen minutes, at the end of which time it was fixed. Leitz oc. 8, obj. 2mm.

Fig. 7. Cross section of vitellarium of animal recovering from desiccation. Animal was kept in an evacuated desiccator for six days, then placed in water for one hour, at the end of which time it was fixed. Leitz oc. 8, obj. 2mm.



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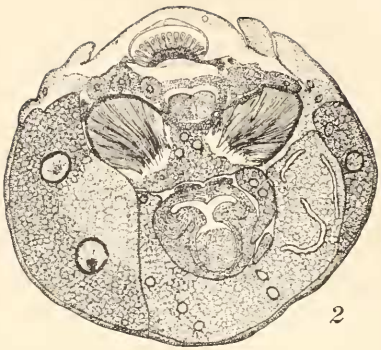

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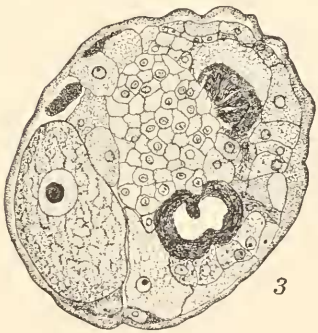

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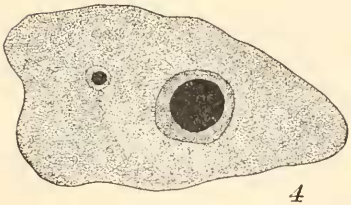

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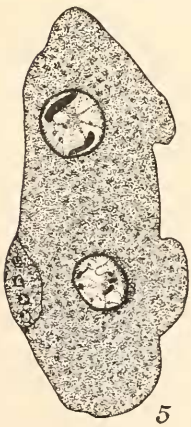

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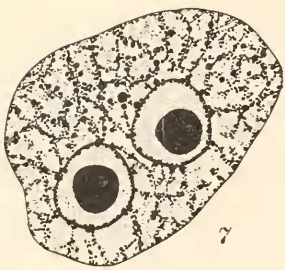

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LOUIS M. HICKERNELL.

PLATE II.

FIG. 8. Section of brain of *P. roseola*. Normal active animal. Leitz oc. 8, obj. 2mm.

FIG. 9. Section of brain of a rotifer which was kept in an evacuated desiccator for fourteen days previous to the time of fixation. Leitz oc. 8, obj. 2mm.

FIG. 10. Section of brain of a rotifer which was dried twenty-four hours, put in water for one hour and then killed. Leitz oc. 8, obj. 2mm.

FIG. 11. Section of foot gland cells from a normal active animal. Leitz oc. 12, obj. 2 mm.

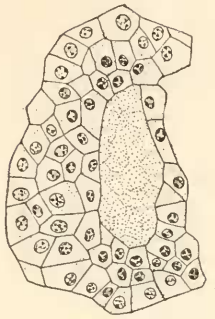
FIG. 12. Section of foot gland ells of a rotifer which was kept in an evacuated desiccator for fourteen days previous to the time of fixation. Leitz oc. 12, obj. 2mm.

FIG. 13. Section of foot gland cells from an animal kept fourteen days in an evacuated desiccator and then placed in water for one and one fourth hours previous to fixation. Leitz oc. 12, obj. 2 mm.

FIG. 14. Section of procambium cell from a germinating corn embryo. Leitz oc. 8, obj. 2mm.

FIG. 15. Section of procambium cell from a partially dried corn embryo. Leitz oc. 8, obj. 2mm.

FIG. 16. Section of procambium cell from a corn embryo dried for a month at room temperature. Leitz oc. 8, obj. 2 mm.



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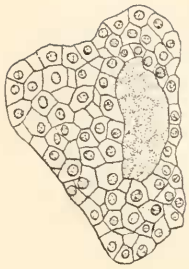

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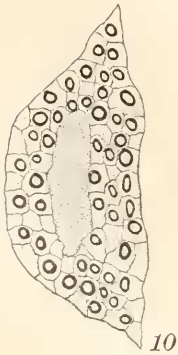

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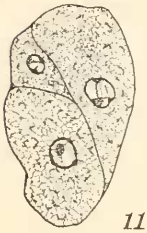

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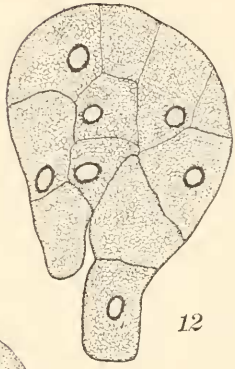

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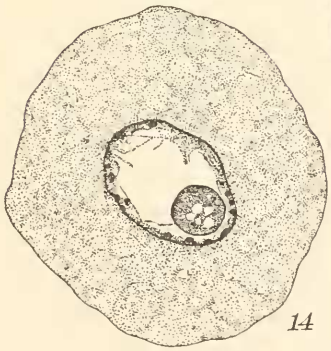

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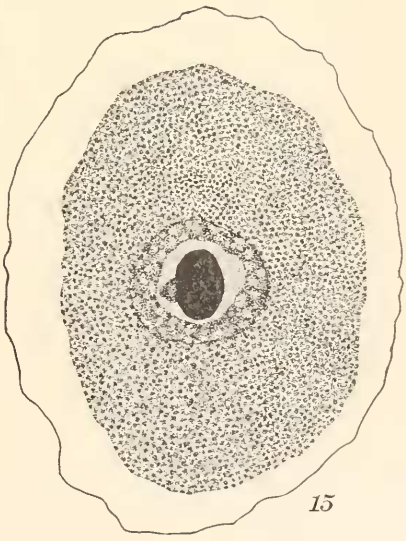

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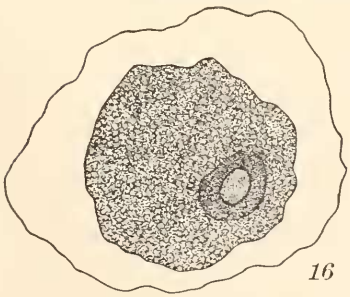

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REGULATION IN VORTICELLA.

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It is a fact well known to students of regeneration that one part of an organism may exert a measurable influence over the growth and development of another. This has been demonstrated for many of the Metazoa under varying forms. The removal of the head of a planarian liberates, as it were, the postjacent tissue, out of which a new head is fashioned. Among macruran crustaceans, the loss of the larger chela of an asymmetrical pair has been shown many times to be succeeded by an accelerated growth of the smaller chela and a subsequent retardation in the regeneration of the lost chela so that, in the presence of the small chela grown large, it remains the smaller of the two. Finally—not to multiply instances needlessly—when a short length of the column, with hydranth, is cut away from the hydroid *Corymorpha*, no development beyond closure of the wound occurs proximally until the hydranth is removed from the distal end. In this respect, the behavior of *Corymorpha* may be contrasted with the behavior of the planarian, since in the latter the presence of the original head on the anterior piece does not inhibit the development of a tail posteriorly. The hydranth in *Corymorpha* appears somehow to inhibit, in short pieces, even the development normally to be expected at the aboral end.

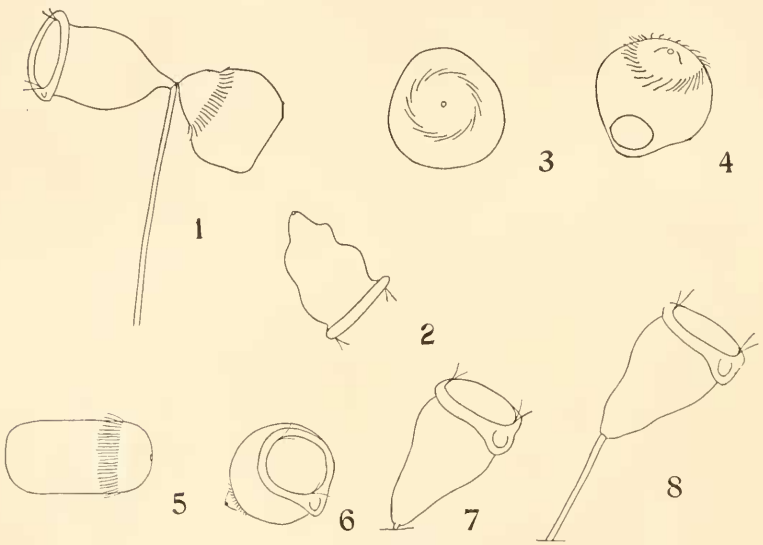
Among the Protozoa, instances of this last sort seem so generally to have escaped record, that we have thought it desirable to describe a similar domination in *Vorticella* sp. of one part over another.

When *Vorticella* divides, the fission plane passes approximately through the center of the organism from oral disk to a point immediately to one side of the contractile stalk. Of the zoöids thus formed, one remains attached to the original stalk, while the other swims away by means of cilia which, during the

last phases of the process of fission, have appeared in a circlet near the aboral pole (Fig. 1).[1]

Why do such cilia not appear on the stalked zoöid?

It may be noticed in the last stages of fission that the zoöid destined to become free retains its connection by a slender protoplasmic strand with the body of the stalked zoöid, not directly with the stalk itself. This fact suggests what has proven to be the correct view, namely, that cilia which would normally develop on every individual are able to show themselves only when sufficiently isolated physiologically from the stalk. Such isolation exists when the connection between the separating zoöids is reduced to a narrow strand.



FIGS. 1–8.

This view was reinforced by the familiar fact that, upon becoming attached to the substrate, the free zoöid gradually loses its cilia as its stalk develops. In the normal life history, then, aboral cilia develop in isolation from the stalk and disappear with the development of the stalk.

The test was applied by cutting a stalked zoöid quite away from the stalk. This was accomplished under a binocular, by

[1] The figures have not been drawn with a camera. Their scale varies somewhat.

means of a sharp dissecting needle. A typical case is shown in Figs. 2–8. Soon after the cutting, the zoöid (Fig. 2) settled down on its oral surface. In an hour, cilia began to push out in a circlet near the aboral pole. They elongated rapidly, and began to beat around the oral-aboral axis (Fig. 3). The oral disk turned in upon itself in the manner characteristic of the normally free zoöid (Fig. 4). One hundred and five minutes after the operation, the zoöid swam away (Fig. 5), indistinguishable in every respect from the normally free form. After five minutes of active locomotion, it came to rest on its aboral end, became attached, and unfolded its oral disk (Fig. 6). At once the stalk began to grow and the aboral cilia to disappear. In ten minutes no aboral cilia were to be seen (Fig. 7). Two hours and a half later, the organism appeared as in Fig. 8.

The development of the stalk appears to be dependent on contact at the aboral end; while the development of aboral cilia is conditioned by physiological isolation from the stalk whether achieved experimentally or by a narrowing of protoplasmic connection in the ordinary course of fission.